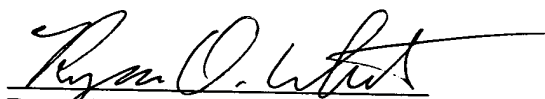


REMARKS

By the present amendment, the specification is amended to correct various matters of form on pages 2, 3 and 8. Additionally, Applicant has canceled Figure 3 and renumbered original Figures 4 and 5 as well as updated the specification accordingly. Support for Figures 3a, 3b and 3c may be found in original Figure 4, while support for Figures 4a, 4b and 4c may be found in original Figure 5. Support for adding the sentence regarding immunodetection of Thy-1,2⁺ T cells in the islets of congenic NOD-N Thy-1,1 mice three weeks after adoptive cell transfer to the paragraph appearing at page 7, lines 5-16, may be found at page 9, lines 21-25 of the original specification as filed. Additionally, claims 7, 9 and 10 are amended to recite IGF-I analogues and claims 1-6 and 8 are cancelled. Furthermore, claims 11-19 are added to more specifically recite the dosage requirements of the IGF-I analogues, while claims 20-27 are added to more specifically recite the sequence identity of the IGF-I analogues. A Version With Markings Showing Changes Made is attached. It is believed that these changes do not involve any introduction of new matter, whereby entry is believed to be in order and is respectfully requested.

Respectfully submitted,



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VERSION WITH MARKING SHOWING CHANGES MADE

In the Specification:

The paragraph appearing at page 2, lines 15-26 is amended as follows:

--Insulin like growth factor-1 (IGF-1), a 70-amino acid peptide structurally related to insulin, is normally considered to be a metabolic hormone which mediates many effects of growth hormone. Prophylactic insulin treatment of NOD mice during the prediabetic phase (5) as well as insulin treatment of the NOD recipients of autoreactive T cells during adult T cell transfer (6) have been shown to prevent and/or delay the onset of diabetes and to reduce the severity of insulinitis. Similar results have been also obtained in BB rats (7,8), which [as] are another animal model of spontaneous autoimmune diabetes. Since insulin is a major antigenic component of the beta cells, it was not clear from these experiments whether insulin protective effects were explained by an antigen-specific unresponsiveness of the immune system, by a direct suppressive effect on T cell function, or by a direct effect on the beta cells.--

The paragraph bridging pages 2 and 3 is amended as follows:

--IGF-I has also shown to have a protective effect against diabetes, [is] in preventing beta cell destruction in subjects which are at high risk of development of diabetes and in the regulation of T cells in subjects which are at high risk of developing diabetes.--

The paragraphs referencing the Figures and appearing at page 3, lines 10-20 are amended as follows:

--[5] The following figures are illustrating the results of the experiments.

Figure 1 Cumulative incidence of diabetes in four independent experiments in mice;

Figure 2 Severity of insulinitis and destructive lesions;

[Figure 3 Immunodetection of Thy-1,2⁺ T cells in the islets of congenic NOD-N Thy-1,1 mouse]

[Figure 4] Figures 3a-3c FACS analysis of Thy-1,2⁺ T cells within the spleen of a congenic NOD-N Thy-1,1 mouse; and

[Figure 5] Figures 4a-4c FACS analysis of Thy-1,2⁺ T cells within the thymus of a congenic NOD-N Thy-1,1 mouse.--

The paragraph appearing at page 7, lines 5-16 is amended as follows:

--Because insulinitis is a T cell phenomenon, we suspected that rhIGF-1 might interfere with the kinetics of the migration of committed T cells to the pancreas. Congenic NOD-N Thy-1,1 males were adoptively transferred with T cells from diabetic NOD Thy-1,2 animals. Diabetes occurred in 3 / 6 mice that had been treated with saline and 0 / 6 mice that had been treated with rhIGF-1, after 3 weeks of treatment. This apparent protective effect was also associated with a decrease in the severity of islet cell infiltrates, which were composed exclusively by T cells from donor origin with no recruitment of host T cells [as shown in figure 3]. More particularly, immunodetection of Thy-1,2⁺ T cells in the islets of congenic NOD-N Thy-1,1 mice three weeks after adoptive cell transfer of diabetes using 7x10⁶ T cells from NOD Thy-1,2 diabetic donors illustrates a severe insulinitis in a control mouse while illustrating a peri-insulinitis in a mouse treated with rhIGF-1. Additionally, [When] when analyzed in individual mice, the number of Thy-1,2⁺ T cells was found to be significantly lower in the spleen of treated mice with rhIGF-1 in comparison with control mice (Table III and [figure 4] Figures 3a, 3b and 3c), although no [significant] significant difference was noted within the thymus ([figure 5] Figures 4a, 4b and 4c).--

The paragraph appearing at page 8, lines 12-32 is amended as follows:

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--The observation of pancreatic glands free from insulitis under rhIGF-1 treatment, suggests another mechanism that occurs prior to the late activation process of infiltrating T cells by eliminating or inactivating the functional properties of autoreactive T cells necessary for beta cell destruction. Recombinant hIGF-1 may exert these effects directly on lymphoid cells, since in vitro suppression of T cell response to concanavalin A or allogeneic stimulation can be achieved in a dose dependent manner (16). Many actions of growth-hormone on the immune system may be mediated by IGF-1 which is also produced by peripheral leukocytes (17). Recent observations suggest that activated T lymphocytes possess receptors for IGF-1 (18-20). In addition, several reports indicate that IGF-1 may influence thymic epithelial cell function in vitro (21) and induce thymocyte replication and differentiation in streptozocin induced diabetic rats (22). Mice which receive 4 mg/kg per day of rhIGF-1 were found to have an increased spleen and thymus weight, due to an increase in the number of lymphocytes in these organs, preferentially T cells from the CD4 phenotype (22). We did not observe any difference in the number of T cells in the lymphoid organs and in the relative contribution of T cell subsets within the spleen, probably because of lower doses of rhIGF-1 used in the present study. Moreover, treatment of diabetic females with rhIGF-1 failed to reduce the capacity of spleen cells to transfer the disease, suggesting that the number and degree of activation of autoreactive T cells were not modified.--

The paragraphs referencing the LEGENDS OF FIGURES and appearing at page 9, lines 12-37 are amended as follows:

--LEGENDS OF FIGURES

Figure 1: Cumulative incidence of diabetes in four independent experiments following adoptive T cell transfer in 24 mice injected twice daily with 10 μ rhIGF-1 (open circles) and 21 control mice injected with saline (closed circles).

Figure 2: Severity of insulinitis and destructive lesions of recipient mice according to treatment with saline (dark columns) or rhIGF-1 (open columns). Results are mean percentages \pm SE from 24 individual mice from two independent experiments.

[Figure 3: Immunodetection of Thy-1,2⁺ T cells in the islets of congenic NOD-N Thy-1,1 mice three weeks after adoptive cell transfer of diabetes using 7×10^6 T cells from NOD Thy-1,2 diabetic donors. Panel A illustrates a severe insulinitis in a control mouse. Panel B represents a peri-insulinitis in a mouse treated with rhIGF-1.]

[Figure 4] Figures 3a, 3b and 3c: FACS analysis of Thy-1,2⁺ T cells within the spleen of a congenic NOD-N Thy-1,1 mouse, three weeks after sub-lethal irradiation and inoculation of Thy-1,2⁺ T cells from diabetic donors. [Panel A] Figure 3a represents the results in a control NOD-N Thy-1,1 mouse. Insulin like growth factor-1 significantly reduced the number of Thy-1,2⁺ in the spleen [(Panel B)] (Figure 3b) in comparison to saline [(Panel C)] (Figure 3c).

[Figure 5] Figures 4a, 4b and 4c: FACS analysis of Thy-1,2⁺ T cells within the thymus of a congenic NOD-N Thy-1,1 mouse, three weeks after sub-lethal irradiation and inoculation of Thy-1,2⁺ T cells from diabetic donors. [Panel A] Figure 4a represents the results in a control NOD-N Thy-1,1 mouse. The effects of rhIGF-1 upon the reconstitution of the thymus after T cell transfer are shown in [Panel B] Figure 4b and are compared to saline injected mouse [(Panel C)] (Figure 4c).--

In the Claims:

Claims 7, 9 and 10 are amended as follows:

7. (Amended) Method for delaying the clinical onset of diabetes by administration of an IGF-I [or analogues thereof] analogue.

9. (Amended) Method for [preventing] reducing the occurrence of beta cell destruction in [subjects which are at] a subject having a high risk of developing diabetes by administration of an IGF-I [or analogues thereof] analogue.

10. (Amended) Method for [regulating] reducing the number of T cells migrating to the spleen in [subjects which are at] a subject having a high risk of developing diabetes by administration of an IGF-I [or analogues thereof] analogue.

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